Biol Trace Elem Res (2012) 150:130–136
DOI 10.1007/s12011-012-9452-3

Effect of High-Dose Nano-selenium and Selenium–Yeast on Feed Digestibility, Rumen Fermentation, and Purine Derivatives in Sheep

Wenjuan Xun · Liguang Shi · Wenbin Yue · Chunxiang Zhang · Youshe Ren · Qiang Liu

Received: 1 March 2012 / Accepted: 11 May 2012 / Published online: 14 June 2012

Abstract The aim of this study was to evaluate the effect of nano-selenium (NS) and yeast–selenium (YS) supplementation on feed digestibility, rumen fermentation, and urinary purine derivatives in sheep. Six male ruminally cannulated sheep, average 43.32±4.8 kg of BW, were used in a replicated 3×3 Latin square experiment. The treatments were control (without NS and YS), NS with 4 g nano-Se (provide 4 mg Se), and YS with 4 g Se-yeast (provide 4 mg Se) per kilogram of diet dry matter (DM), respectively. Experimental periods were 25 days with 15 days of adaptation and 10 days of sampling. Ruminal pH, ammonia N concentration, molar proportion of propionate, and ratio of acetate to propionate were decreased ($P<0.01$), and total ruminal VFA concentration was increased with NS and YS supplementation ($P<0.01$). In situ ruminal neutral detergent fiber (aNDF) degradation of *Leymus chinensis* ($P<0.01$) and crude protein (CP) of soybean meal ($P<0.01$) were significantly improved by Se supplementation. Digestibilities of DM, organic matter, crude protein, ether extract, aNDF, and ADF in the total tract and urinary excretion of purine derivatives were also affected by feeding Se supplementation diets ($P<0.01$). Ruminal fermentation was improved by feeding NS, and feed conversion efficiency was also increased compared with YS ($P<0.01$). We concluded that nano-Se can be used as a preferentially available selenium source in ruminant nutrition.

Keywords Selenium–yeast · Nano-selenium · Sheep · Feed digestibility · Rumen fermentation · Purine derivatives

Introduction

Se is an essential trace element, and its importance for animal health and productivity has been well confirmed. Se has known to be involved in enzyme activity and preventing oxidative damage to body tissue [1]. Se is also associated with protein fractions in microorganisms and plants [2]. Se has a very narrow margin between its lowest acceptable level of intake and its toxicity. The toxicity of Se depends on the chemical form, species, physiological state, nutrition, and dietary interactions [3, 4].

Diets for ruminants are almost exclusively of plant origin and the Se concentration within plants can be varied remarkably in China and other countries [5, 6]. The deficiency of Se always causes great losses in livestock. Thus, addition of Se to animal diets is required around the low Se region. Inorganic informs, typically sodium selenite (Na_2SeO_3) or selenate (Na_2SeO_4) and organic Se such as Se-enriched yeast (YS), are two principal forms used in ruminants [7] and EEC (2006) [8]. Prior studies have confirmed that organic Se is more effective than inorganic Se in ruminants [9–15] and inorganic Se is more toxic than organic Se [16]. Recently, elemental nano-Se caused great interest in the high bioavailability and low toxicity [17–19]. Shi et al.

Wenjuan Xun and Liguang Shi contributed equally to this work.

W. Xun · W. Yue (✉) · C. Zhang · Y. Ren · Q. Liu
College of Animal Sciences and Veterinary Medicines, Shanxi Agricultural University,
Taigu, Shanxi 030801, People's Republic of China
e-mail: wb-yue@126.com

W. Yue
e-mail: wenbinyue991@yahoo.com

W. Xun · L. Shi
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences/Key Laboratory of Crop Gene Resources and Germplasm Enhancement in Southern China, Ministry of Agriculture,
Danzhou, Hainan 571737, People's Republic of China

(2011) reported addition of nano-Se to sheep feedstuffs could improve rumen fermentation and feed utilization. However, little is known about the influence of different Se sources at high dose on fermentation characteristic and nutrient digestibility [20].

Thus, the objectives of this research were to investigate the effects of feeding high-dose YS and NS on feed digestibility, rumen fermentation, and excretion of purine derivatives in sheep.

Materials and Methods

Chemicals

The Se-enriched yeast was purchased from Angel Yeast Co., Ltd., China. The concentration was 1,000 mg/kg of Se. Nano-Se was purchased from Shanghai Stone Nano-Technology Port Co., Ltd., China, and concentration was also 1,000 mg/kg of Se.

Animals, Diets, and Experimental Design

Six Dorset sheep×Small Tail Han×Tan sheep, average 43.32±4.8 kg of body weight (BW), were ruminally cannulated and used in a replicated 3×3 Latin square experimental design with three 25-day periods (15-day adaptation and 10-day sampling collection each period). The three treatments were a basal diet (control), a basal diet supplemented with 4 g YS (provide 4 mg Se) per kilogram of diet dry matter (DM), and a basal diet supplemented with 4 g NS (provide 4 mg Se) per kilogram of dry matter (DM). The supplement of YS or NS was mixed into concentrate in the diets. Sheep were fed twice daily (07:00 and 19:00 h) at maintenance nutrition requirements with a basal diet consisting of 300 g/kg (dry matter) DM of basal concentrates and 700 g/kg DM of forage. The proximate composition of basal diet is shown in Table 1. Sheep were placed in metabolic cages individually and fresh water was freely available during the experimental period.

Table 1 The ingredients and chemical composition of the basal diet (g/kg dry matter)

Ingredients	
Alfalfa hay, ground	368
Cracked corn	168
Corn stalk, ground	290
Wheat bran	22
Soybean meal	58
Sunflower meal	29
Salt	5
Calcium phosphate	3
Minerals and vitamin premix[a]	2
Chemical composition[b]	
Dry matter	856±7
Crude protein	122±3
Acid detergent fiber	285±5
Neutral detergent fiber	437±5
Calcium	6.5±0.04
Phosphorus	2.4±0.02
Selenium (mg/kg of DM)	0.044
Metabolizable energy (MJ/kg)	9.82

[a] Contained 45 ppm Zn, 40 ppm Mn, 1.0 ppm I, 1.0 ppm Co, 45 ppm Fe, 20 ppm Cu, 2,500 IU/kg of vitamin A, 400 IU/kg of vitamin D, and 200 IU/kg of vitamin E

[b] Analyzed values except metabolizable energy

Digestibility in the Total Tract

A digestion trial was conducted with all sheep on days 11–20 of each period. Sheep was dosed via ruminal cannula with 1 g chromic oxide twice daily at 07:00 and 19:00 h for 10 days. The initial 5 days were used for uniform chromic oxide excretion and next five sampling days were used for collection of feces. Fecal pellets were collected from rectum at 07:00 and 19:00 h, and representative samples of the feces were pooled by sheep for each period. After drying in an oven at 60 °C, the samples were ground to pass a 1-mm sieve for chemical analyses. Dry matter excreted in feces was evaluated by dividing chromium input by chromium concentration in the feces. Excretion of other nutrients in the feces was evaluated by multiplying DM flow by their concentration in fecal DM.

Ruminal pH and Fermentation

Samples of rumen fluid were collected through the cannula at 0 h (i.e., just before feeding in the morning), 3, 6, and 9 h after feeding on days 23 and 24 of each collection period for pH, ammonia-N, and volatile fatty acids (VFAs) determination. Ruminal pH was immediately measured using an electric pH meter (Sartorius Basic pH Meter PB-20; Sartorius AG, Goettingen, Germany).

In Situ Ruminal Degradability

Ruminal degradability of soybean meal and *Leymus chinensis* was determined using nylon bag technique on days 16–18 of each experimental period. Soybean meal and *Leymus chinensis* were ground through a 2.5-mm screen, and about 3.5 g of *L. chinensis* and 4.5 g of soybean meal were weighed into 6 cm×10 cm nylon bags made of monofilament Pecap polyester (Guangzhou Minyuan Business Co., Ltd., Guangdong, China). The bags were incubated in the

rumen of each sheep for 0, 6, 12, 24, 48, and 72 h. As soon as the bags were removed from the rumen, they were rinsed in cold water until they were clean, and then dried in an oven at 65 °C for 12 h, 105 °C for 24 h. Residual DM of bags were ground to pass a 1-mm screen for chemical analysis. Ruminal nutrient degradability was fitted to the exponential model:

$$y = a + b\left(1 - e^{-c(t-L)}\right),$$

for $t>L$ [21], where a is the soluble fraction, b is the slowly degradable fraction, c is the fractional degradation rate constant at which b is degraded, L is the lag time (h), and t is the time of incubation (h). The non-linear parameters "a", "b", and "c" were estimated using the non-linear regression procedure of SAS (1996). Effective degradability (ED) of feeds in the rumen were calculated as $\mathrm{ED} = a + [(b \times c)/(c + k)]$ [22], where k was the passage rate of the digesta from the rumen and was 0.02/h for *L. chinensis* and 0.052/h for soybean meal according to our measurements.

Urine Collection and PD Measurements

Total collections of urine were conducted for 10 days (from day 16 to 25). Urine volume was measured daily and collected into buckets containing approximately 10 mL of 10 % H_2SO_4 to reduce pH below 3 for prevention bacteria destruction of purine derivatives (PD). At the end of the collection days, 20-mL urine samples were diluted to 100 mL with distilled water, then sub-sampled and stored at −20 °C for analysis of allantoin, uric acid, xanthine, and hypoxanthine. The relationship between microbial purines absorbed (X, mmol/day) and the purine derivatives (PD) excreted (Y, mmol/day) was estimated using the equation described by Chen and Gomes [23]:

$$Y = 0.84X + \left(0.150W^{0.75}e^{-0.25X}\right)$$

Laboratory Analysis

All dried samples were ground through a mill to pass a 1-mm screen and later analyzed for dry matter (DM, ID no. 934.01), organic matter (OM, ID no. 942.05), and crude protein (CP, ID no. 984.13) according to methods of AOAC [24]. Neutral detergent fiber (aNDF, with heat-stable alpha amylase and sodium sulfite, and expressed inclusive of residual ash) and acid detergent fiber (ADF) were determined according to Van Soest et al. [25]. Ruminal VFA was determined using gas chromatography (GC102AF; Shanghai Specialties Ltd., China). The concentrations of ammonia N (NH_3–N) was determined by the method of AOAC [24]. Allantoin and uric acid in urine was determined according to the procedure of IAEA (1997).

Statistical Analyses

Data on digestibility were analyzed using the mixed model procedure of SAS (Proc Mixed) [26] to test the effects of square, period within square, animal within square, and treatment. The treatment was as a fixed effect; square, period within square, and animal within square were as random effects. Rumen fermentation parameters were also analyzed using the same mixed model but with time included as a repeated measures. Relationships between measured variables were analyzed using linear correlation procedure of SAS. Effects of factors were considered significant at P <0.05.

Results

Digestibility in the Total Tract

As shown in Table 2, digestibility of DM, organic matter (OM), crude protein (CP), ether extract (EE), aNDF, and ADF in the total tract were higher in Se supplemented animals than in control sheep (P<0.01), and also with significantly (P<0.01) higher values in NS group compared to SY group.

Ruminal pH and Fermentation

Ruminal fermentation characteristics are shown in Table 3. Mean ruminal pH, ammonia N content, molar proportion of propionate, and the ratio of acetate to propionate were lower for NS supplemented sheep compared to the control and YS treatment (P<0.01). Total ruminal VFA concentration was higher (P<0.01) in YS group. Molar proportions of acetate and butyrate were not affected by Se supplementation (P>0.05).

Effective Ruminal Degradability

Table 4 showed the in situ ruminal digestion kinetics and effective degradability (ED) of *L. chinensis* and soybean meal. For *L. chinensis*, the soluble fraction, slowly degradable fraction, fractional degradation rate, and the ED of *L. chinensis* DM and aNDF were higher for NS than YS and control treatments (P <0.01), and the YS was higher than control treatments (P<0.01).

For soybean meal, the soluble fraction, slowly degradable fraction, and ED of DM were higher for NS compared to the YS and control treatments (P<0.01), and YS was higher than control treatments (P<0.01). However, fractional degradation rate in NS was the lowest among treatments (P<0.01). Similarly, the soluble fraction, slowly degradable fraction, fractional degradation rate, and the ED of CP were

Table 2 Effects of high-dose nano-selenium (NS) and selenium–yeast (SY) supplementation on nutrient digestibility in the total tract of sheep

Item	Treatments[a]			SE	*P* value
	Control	NS	YS		
Dry matter	0.616a	0.694c	0.652b	0.018	0.011
Organic matter	0.636a	0.708c	0.663b	0.011	0.010
Crude protein	0.615a	0.672c	0.639b	0.009	0.030
Ether extract	0.523a	0.577b	0.543a	0.008	0.048
Neutral detergent fiber	0.514a	0.614c	0.573b	0.015	<0.001
Acid detergent fiber	0.500a	0.584c	0.557b	0.013	<0.001

Means in the same row with different letters differ significantly ($P<0.05$)

[a]Control (without nano-Se or selenium–yeast), NS and YS with 4 mg/kg DM Se, respectively

higher in NS compared with other treatments ($P<0.01$), and YS was higher than control treatments ($P<0.01$).

Urinary Purine Derivatives

Urinary purine derivatives are shown in Table 5. Daily urinary excretion of uric acid, xanthine, and hypoxanthine were not affected by NS and YS addition ($P>0.05$). However, urinary excretion of allantoin and PD were higher in NS and YS treatments than control treatment ($P<0.01$), and urinary excretion of PD in NS treatment was higher than YS treatment ($P<0.01$).

Discussion

Ruminal Fermentation

Supplementation of NS or YS in sheep switched rumen fermentation pattern from acetate to propionate as shown by the reduction in ratio of acetate to propionate. The decrease of the ratio of acetate to propionate and the increase of total VFA concentration resulted from the increased propionate concentration. It is suggested that Se supplementation in sheep diets particularly improved ruminal microbial activity in propionate production. In the present study, mean ruminal pH was within the optimum range for cellulolytic bacteria activity [27]. The increase in the total VFA and propionate production and reduction in the mean ruminal pH by high dose NS or YS supplementation in our research agree with the results of Shi et al. (2011) who obtained higher VFA concentration and lower pH in the sheep fed with the basal diet supplemented with 3 g of nano-Se/kg DM (provide 3 mg Se) in sheep [20]. The similar results were also found in cows [28] and in lambs [29]. However, Serra et al. showed concentration of total VFA and propionate decreased slightly by administration 0.2 mg Se (Na_2SeO_3 or Na_2SeO_4) per kilogram of DM in sheep [30]. The conflicting results may ascribe the conversion of inorganic Se to insoluble forms in the rumen, thus decreasing its availability. In the present study, the total VFA concentration was higher in NS than YS treatment. Thus, we concluded absorption and availability of Se was closely related to chemical form of Se in ruminant. Zhang et al. (2008) noted nano-Se possessed a higher bioavailability because of its excellent catalytic efficiency, low toxicity, and strong adsorbing ability [19]. All these novel properties of nano-Se and higher bioavailability may explain increased rumen fermentation by NS supplementation compared with YS.

Apparent Digestibility in the Total Tract and In Situ Ruminal Degradability

The increase of in situ ruminal ED of *L. chinensis* DM and NDF was consistent with higher ruminal total VFA

Table 3 Effects of high-dose nano-selenium (NS) and selenium–yeast (SY) supplementation on ruminal pH and fermentation in sheep

Item	Treatments[a]			SE	*P* value
	Control	NS	YS		
pH	6.79c	6.34a	6.57b	0.065	<0.001
Ammonia N (mg/100 mL)	11.05c	8.35a	9.79b	0.039	<0.001
Acetate (A) (mol/100 mol)	60.52	58.42	59.03	0.425	0.118
Propionate (P) (mol/100 mol)	18.23a	21.38c	19.56b	0.474	<0.001
Butyrate (mol/100 mol)	6.01	5.89	5.92	0.024	0.075
A/P	3.32c	2.73b	3.02b	0.085	<0.001
Total VFA (mM)	91.13a	96.41c	94.19b	0.774	<0.001

Means in the same row with different letters differ significantly ($P<0.05$)

[a]Control (without nano-Se or selenium-yeast), NS and YS with 4 mg/kg DM Se, respectively

Table 4 In situ ruminal digestion kinetics and effective degradability (ED) of *Leymus chinensis* and soybean meal

Item	Treatments[a]			SE	*P* value
	Control	NS	YS		
Leymus chinensis					
DM					
a[b]	0.135a	0.179c	0.152b	0.007	0.014
b	0.532a	0.802c	0.683b	0.039	<0.001
c (/h)	0.011a	0.020c	0.016b	0.001	0.003
ED	0.320a	0.582c	0.457b	0.038	<0.001
aNDF					
a[b]	0.041a	0.073c	0.059b	0.005	<0.001
b	0.605a	0.926c	0.802b	0.047	<0.001
c (/h)	0.010a	0.017c	0.014b	0.002	<0.001
ED	0.247a	0.503c	0.398b	0.038	<0.001
Soybean meal					
DM					
a[b]	0.170a	0.284c	0.219b	0.017	<0.001
b	0.550a	0.657c	0.630b	0.017	<0.001
c (/h)	0.052b	0.027a	0.035b	0.004	<0.001
ED	0.445a	0.509c	0.471b	0.010	0.001
CP					
a[b]	0.130a	0.193c	0.165b	0.009	0.001
b	0.804a	0.907c	0.852b	0.015	0.001
c (/h)	0.017a	0.025c	0.021b	0.001	0.003
ED	0.331a	0.490c	0.413b	0.023	<0.001

Means in the same row with different letters differ significantly ($P<0.05$)

[a] Control (without nano-Se or selenium-yeast), NS and YS with 4 mg / kg DM Se, respectively

[b] Parameters were calculated from the fitted equation $y = a + b \times (1 - e^{-c(t-L)})$ for $t>L$, where y=percentage of DM disappearance from the nylon bag at time t, a=soluble fraction, b=slowly degradable fraction, c=fraction rate constant at which b is degraded, L=lag time (h), and t=time of incubation (h). Effective degradability (ED) was calculated using equation $a + bc/(c + k)$, where k=0.02/h for *Leymus chinensis* and 0.052 /h for soybean meal, respectively

concentration and increased nutrient digestibility in the total tract in NS treatment compared to control and YS. Our results are in accordance with that published by Shi et al. (2011) who found in situ ruminal ED of *L. chinensis* DM and NDF was increased by supplementation with 3 g nano-selenium (provide 3 mg Se) per kilogram of DM in sheep [20]. However, the results are in contrast with the report by Serra et al. who found that supplementation of sheep feed with 0.2 mg Se (Na_2SeO_3 and Na_2SeO_4, per kilogram of DM) had no effect on digestibility of NDF [30]. The results showed addition 4 g/kg dietary DM NS could significantly increase growth and activity of cellulolytic bacteria compared to YS, and thus improved rumen fermentation. This allowed us to conclude that different metabolic ways exist between inorganic Se and organic Se in rumen. Further studies are necessary to clarify the metabolic mechanisms of different Se forms.

The increase of in situ ruminal ED of soybean meal DM and CP by NS or YS supplementation was also in line with the prior study that digestibility of crude protein was significantly increased by supplementation with 3 mg/kg dietary DM Se (nano-Se) in sheep [20]. The similar results were also found in rats [31], in pigs [32], and in dairy cows [28]. In the present study, the digestibility of CP and in situ ruminal ED of soybean meal CP were higher in NS than YS treatment. The results suggest nano-Se supplementation could significantly increase activity of protein-decomposing bacteria and promote proteolytic digestive enzymes activity.

Nitrogen Metabolism and Urinary Purine Derivatives Excretion

Se supplementation could increase rumen microbial population and activity [18, 33, 34]. The reduction of ammonia N concentration by NS or YS supplementation could be due to an enhanced growth of ruminal microbial populations which increased the ammonia N consumption. The increased urinary excretion of PD (Table 5) suggested that Se supplementation may increase the microbial protein production in the rumen. Besides, Russell et al. observed that cellulolytic bacteria obtain their N exclusively from ammonia N [35]. One can suggest that NS or YS supplementation may increase the ruminal microbial protein synthesis as effective ruminal degradability and the nutrient digestion in the total tract was improved.

Table 5 Effects of high-dose nano-selenium (NS) and selenium–yeast (SY) supplementation on urinary purine derivatives in sheep

Item	Treatments[a]			SE	*P* value
	Control	NS	YS		
Urinary excretion (mmol/day)					
Allantoin	11.230a	15.143b	14.597b	0.647	0.001
Uric acid	3.137	3.233	3.170	0.021	0.469
Xanthine and hypoxanthine	0.247	0.273	0.257	0.007	0.546
Total PD	14.513a	18.530c	17.743b	0.615	<0.001

Means in the same row with different letters differ significantly ($P<0.05$)

[a]Control (without nano-Se or selenium–yeast), NS and YS with 4 mg/kg DM Se, respectively

Conclusions

Supplementation of NS and YS in sheep increased ruminal VFA concentration and switched rumen fermentation pattern from acetate to propionate. Nutrients digestibility in the total tract, in situ ruminal aNDF degradation of *L. chinensis* and CP of soybean meal, and urinary excretions of PD were also improved by Se supplemented. Feeding 4 g nano-Se per kilogram of dietary DM could significantly improve feed utilization and rumen fermentation compared with yeast–Se.

Acknowledgments This work was supported by the Earmarked Fund for Modern Agro-industry Technology Research System and National Nonprofit Institute Research Grant of CATAS-TCGRI (1630032012022). The authors thank the staff of the Key Laboratory of Animal Breeding and Genetics & Reproduction, Shanxi Agriculture University.

References

1. Neve J (2002) Selenium as a "nutraceutical": how to conciliate physiological and supra nutritional effects for an essential trace element. Curr Opin Clin Nutr Metab Care 5:659–663
2. Whanger PD (2002) Selenocompounds in plants and animals and their biological significance. J Am Coll Nutr 21(3):223–232
3. Shin Y, Blackwood JM, Bae IT, Arey BW, Exarhos GJ (2007) Synthesis and stabilization of selenium nanoparticles on cellulose nanocrystal. Mater Lett 61:4297–4300
4. Li H, Zhang J, Wang T, Luo W, Zhou Q, Jiang G (2008) Elemental selenium particles at nano-size (Nano-Se) are more toxic to Medaka (*Oryzias latipes*) as a consequence of hyper-accumulation of selenium: a comparison with sodium selenite. Aquat Toxicol 89 (4):251–256
5. Surai PF (2006a) Selenium in food and feed: selenomethionine and beyond. In: Selenium in nutrition and health. Nottingham University Press, Nottingham, pp 151–212
6. Juniper DT, Phipps RH, Ramos-Morales E, Bertin G (2008) Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in beef cattle. J Anim Sci 86(11):3100–3109
7. Register F (2002) Food additive permitted in feed and drinking water: selenium yeast. Federal Reg 67(137):46850–46851
8. EEC (2006) Commission Regulation (EC) No. 1750/2006 of 27 November 2006 concerning the authorisation of selenomethionine as a feed additive
9. Schrauzer GN (2003) The nutritional significance, metabolism and toxicology of selenomethionine. Adv Food Nutr Res 47:73–112
10. Weiss WP (2003) Selenium nutrition of dairy cows: comparing responses to organic and inorganic selenium forms. In: Lyons T. P. (Ed.) Nutritional technology in the feed and food industries. Proceedings of Alltech's 19th Annual Symposium, pp 333–343.
11. Weiss WP, Hogan JS (2005) Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. J Dairy Sci 88 (12):4366–4374
12. Qin S, Gao J, Huang K (2007) Effects of different selenium sources on tissue selenium concentrations, blood GSH-Px activities and plasma interleukin levels in finishing lambs. Biol Trace Elem Res 116:91–102
13. Heard JW, Stockdale CR, Walker GP, Leddin CM, Dunshea FR, McIntosh GH, Shields PM, McKenna A, Young GP, Doyle PT (2007) Increasing selenium concentration in milk: effects of amount of selenium from yeast and cereal grain supplements. J Dairy Sci 90(9):4117–4127
14. Pechova A, Misurova L, Pavlata L, Dvorak R (2008) Monitoring of changes in selenium concentration in goat milk during short-term supplementation of various forms of selenium. Biol Trace Elem Res 121(2):180–191
15. Kumara N, Garga AK, Dassa RS, Chaturvedi VK, Mudgal V, Varshneyc VP (2009) Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. Anim Feed Sci Technol 153:77–87
16. Doucha J, Lívanský K, Kotrbácek V, Zachleder V (2009) Production of Chlorella biomass enriched by selenium and its use in animal nutrition: a review. Appl Microbiol Biotechnol 83:1001–1008
17. Xu BH, Xu ZL, Xia MS (2003) Effect of nano red elemental selenium on GPx activity of broiler chick kidney cells in vitro. Wuhan Univ J Nat Sci 8(4):1167–1172
18. Wang H, Zhang J, Yu H (2007) Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. Free Radic Bio Med 42:1524–1533
19. Zhang JS, Wang XF, Xu TW (2008) Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with Se–methylselenocysteine in mice. Toxicol Sci 101:22–23
20. Shi LG, Xun WJ, Yue WB, Zhang CX, Ren YS, Liu Q, Wang Q, Shi L (2011) Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. Anim Feed Sci Technol 163:136–142
21. McDonald I (1981) A revised model for the estimation of protein degradability in the rumen. J Agric Sci 96:251–252
22. Ørskov ER, McDonald I (1979) The estimate of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci 92:499–503
23. Chen XB, Gomes MJ (1992) Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—an overview of the technical details. Occasional Publication, International Feed Resources Unit. Rowett Research Institute, Aberdeen, p 21
24. AOAC (1990) Official Methods of Analysis, vol. 1., 15th ed. Association of Official Analytical Chemists, Arlington, pp 69–90
25. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597
26. SAS User's Guide (1996) Statistics Version, 7th edn. SAS Institute, Inc., Cary
27. Russell JB, Wilson DB (1996) Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J Dairy Sci 79:1503–1509
28. Wang C, Liu Q, Yang WZ, Dong Q, Yang XM, He DC, Zhang P, Dong KH, Huang YX (2009) Effects of selenium yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. Live Sci 126:239–244
29. Naziroglu M, Aksakal M, Cay M, Celik S (1997) Effects of vitamin E and selenium on some rumen parameters in lambs. Acta Vet Hung 45:447–456
30. Serra AB, Nakamura K, Matsui T, Harumoto T, Fujihara RI (1994) Inorganic selenium for sheep: II. Its influence on rumen bacterial yield, volatile fatty acid production and total tract digestion of timothy hay. J Anim Sci 7:91–96
31. Ewan RC (1976) Effect of selenium on rat growth, growth hormone and diet utilization. J Nutr 106:702–709

32. Chaudhary M, Garg AK, Mittal GK, Mudgal V (2009) Effect of organic selenium supplementation on growth, Se uptake, and nutrient utilization in guinea pigs. Biol Trace Elem Res 133:217–226
33. Mihaliková K, Gresaková L, Boldizarová K, Faix S, Leng L, Kisidayová S (2005) The effects of organic selenium supplementation on the rumen ciliate population in sheep. Folia Microbiol 50:353–356
34. Faixová Z, Faix Š, Leng L, Váczi P, Maková Z, Szabóová R (2007) Haematological, blood and rumen chemistry changes in lambs following supplementation with Se–yeast. Acta Vet Brno 76:3–8
35. Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ (1992) A net carbohydrate and protein system for evaluating cattle diets. J Anim Sci 70:3551–3561